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Monoseeding improves stand establishment through regulation of apical hook formation and hypocotyl elongation in cotton

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ABSTRACT

Cluster seeding is the dominant sowing pattern of cotton in the Yellow River valley of China, but it expends a large amount of seeds and usually results in slender and long-legged seedlings. The subsequent thinning and final singling of seedlings in cluster seeding are also time-and labor-intensive. Monoseeding may be an alternative sowing pattern to solve these problems. In this study, monoseeding, double seeding and cluster seeding treatments were established by sowing one, two and ten seeds each hill to study their effects on emergence, stand establishment and seedcotton yield as well as the underlying physiological and molecular events during emergence. Results showed that monoseeding, double seeding and cluster seeding were not significantly different in seed emergence rate. But in monoseeding pattern, the stand establishment rate and seedling hypocotyl diameter were increased by 16.3–21.2% and 29.2–34.3%, respectively, while the percentage of seedlings with shell attached, disease incidence and hypocotyl length were reduced by 82.2–91.5%, 36.6–37.2% and 26.9–35.8% respectively when compared with those of cluster seedling. These results indicated that monoseeding not only produced stronger seedlings than double or cluster seeding but also improved stand establishment. Additionally, the apical hook angle of seedlings before emergence under monoseeding was 69.1–71.1% smaller than that under cluster seeding, suggesting improved apical hook formation under monoseeding. The ethylene, IAA, and GA contents in seedlings under monoseeding were higher but the JA content was lower than those under cluster seeding during emergence, suggesting that ethylene, IAA, and GA promoted but JA inhibited the apical formation of seedlings. The increased ethylene content under monoseeding was possibly due to the increased expression of ethylene biosynthesis gene *ACO1*. Monoseeding also increased the expression of apical hook formation promoting genes *COP1* and *HLS1*, but decreased the expression of hook formation inhibiting gene *ARF2*. The expression of hypocotyl elongation related gene *ERF1* under monoseeding increased relative to cluster seeding, which might be an important reason for the shorter and thicker hypocotyl formation under monoseeding than cluster seeding. Monoseeding considerably reduced seeding rate and labor input for thinning and singling of seedlings but produced comparable cotton yield to double or cluster planting. The results suggested that mononseeding improved stand establishment through regulating the expression of apical hook formation- and hypocotyl elongation-related genes as well as changes of some endogenous phytohormones. It was also suggested that monoseeding is a promising seeding pattern to reduce seed and labor inputs without sacrificing yield for cotton production in the Yellow River valley of China and other areas with similar ecology.

1. Introduction

Terrestrial flowering plants often drop their seeds under soil or litter, which serves to protect the propagation process from hostile conditions such as cold temperatures and/or predators (Bentsink and Koornneef, 2008; Vaistij et al., 2013). When the environment becomes favorable for growth, seeds sense the changes and begin the process of germination (Penfield et al., 2005). To grow in the dark, seedlings adopt a developmental strategy known as skotomorphogenesis, which is characterized by long hypocotyls, small and closed cotyledons, and curved apical hooks, an optimal shape to vigorously grow toward the surface (McNellis and Deng, 1995; Von Arnim and Deng, 1996; Chen et al., 2004; Leivar et al., 2008). The apical hook of dark-grown seedlings develops after seed germination to minimize the damage to the

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shoot apical meristem in its way through the soil to reach the light in dicotyledonous plants (Shen et al., 2016).

The *HOOKLESS 1* (*HLS1*) gene encodes an N-acetyltransferase which regulates apical hook formation in Arabidopsis, and the *hls1* mutant fails to form the apical hook and emerge from the soil (Lehman et al., 1996; Shen et al., 2016). HLS1 represents a central integrator of environmental factors and endogenous hormone signals in controlling apical hook formation. Ethylene and gibberellins (GAs) increase *HLS1* expression and promote apical hook formation (An et al., 2012), whereas light and jasmonate (JA) decrease *HLS1* expression and inhibit apical hook formation (Li et al., 2004; Zhang et al., 2014; Zhu, 2014).

Ethylene plays vital roles in physiological processes throughout the plant life cycle, including seedling emergence, cotyledon greening, and leaf senescence (Bleecker and Kende, 2000). The most dramatic effect imposed by ethylene on typical dark-grown seedlings is the "triple response", which is characterized by inhibited root elongation, shortened but thickened hypocotyls by increasing the expression of *ERF1*, and exaggerated apical hook by increasing the expression of *HLS1* gene (Guzman and Ecker, 1990; Benavente and Alonso, 2006; Zhong et al., 2014; Shen et al., 2016). Exaggerated hook is observed in dominant mutants that display elevated levels of ethylene and reduced hook curvature is observed in mutants that fail to increase ethylene biosynthesis (Guzman and Eckert, 1990; Woeste et al., 1999, Vogel et al., 1998a; Vogel et al., 1998b). The expression of ethylene biosynthesis gene ACC oxidase 1 (*ACO1*) is higher in the inner than outer side of the hook during hook formation.

The apical hook is formed as the result of localized cell division and asymmetric cell growth at opposite sides of the apical portion of the hypocotyl. The hook is formed and maintained when the growth rate of the cells at the inner is slower than the outer sides of the hypocotyl (Raz and Ecker, 1999). The establishment of hook structure involves a local accumulation auxin at the inner side of the hook (Mazzella et al., 2014). Mutations in some IAA biosynthetic genes inhibit hook development (Mazzella et al., 2014). GA enhances apical hook formation by promoting cell elongation and cell division at the outer side of the hook and promoting *HLS1* expression (Vriezen et al., 2004; Gallego-Bartolomé et al., 2011; An et al., 2012). Ethylene promotes auxin synthesis, transporting and signaling at the inner sides of apical hooks (Zadnikova et al., 2010; Mazzella et al., 2014). ARF2 is a transcription factor that binds specifically to a DNA sequence present in auxin-responsive promoter elements and represses auxin-induced expression (Tiwari et al., 2003). But, ARF2, which can be inhibited by HLS1, is a repressor of auxin action in apical hook formation (Li et al., 2004; Mazzella et al., 2014).

Light is one of the most influential external stimuli controlling plant development and growth. When seeds germinate buried in the darkness of the soil, light exposure initiates the transition from skotomorphogenesis to photomorphogenesis following by apical hook opening and converting etioplasts to chloroplasts, which, in turn, enable the plants to gain photoautotrophic ability (Kami et al., 2010; Mazzella et al., 2014). Constitutive photomorphogenic 1 (COP1) is a central repressor of light-induced plant morphogenetic changes, which functions as a master switch of light signaling pathway by maintaining skotomorphogenesis and repressing photomorphogenesis (Deng et al., 1991; Osterlund et al., 1999; Lau and Deng, 2012). Recent studies show that COP1 is a key component that senses changes in light fluence to correctly modulate the levels of EIN3 protein during the process of emergence (Shi et al., 2016). Mutation in COP1 causes severe defects in penetrating soil because the expression of *HLS1* gene was decreased and the apical hook opens before emerged from the soil (Shi et al., 2016).

During germination and emergence of soil-buried seeds, the mechanical pressure from top-soil induced ethylene biosynthesized in the seedlings and promoted hook formation through regulated EIN3, ERF1, PIF3, and HLS1 (Shen et al., 2016; Zhong et al., 2014). On the other way, COP1 could directly target EBF1/2, upstream of EIN3, to regulate seedling soil emergence under subterranean darkness (Zhong et al., 2014; Shi et al., 2016). Apical hook has an important role in facilitating seed coat removal in some dicotyledonous species like *Gossypium hirsutum* L. under the mechanical pressure during emerging from the soil (Shichijo et al., 2010).

Cotton belongs to the dicotyledonous plant species, and good stand establishment is the basis for obtaining high yield and fine quality of cotton. Cluster seeding (several seeds per hill) or seeding with a large amount of seeds are traditional measures to foster full emergence and stand establishment; however, it usually results in slender and long-legged seedlings, and the thinning and final singling of seedlings consumes additional labor inputs (Dai et al., 2017). Monoseeding is a relatively light and simplified cultivation alternative, in which the traditional thinning and final singling of seedlings after emergence is eliminated (Dai et al., 2014a,b). However, it is unclear if and how monoseeding can achieve good stand establishment of cotton in field conditions. Thus, we conducted the study with the objectives to determine: 1) the effect of monoseeding on apical hook formation and stand establishment of cotton; 2) the underlying physiological and molecular events involved in hook formation and hypocotyl elongation; 3) the effect of monoseeding on seedcotton yield, yield components and the labor inputs.

2. Materials and methods

2.1. Experimental design

Field experiment was conducted from 2016 to 2017 at the Shandong Cotton Experimental Station, Linqing (115°42′E, 36°61′N), Shandong, China. A commercial Bt (*Bacillus thuringiensis*) transgenic cotton (*Gossypium hirsutum* L.) cultivar K836 was used in the experiment. Acid-delinted seeds (percentage germination ≥ 95%) treated with imidacloprid (Gaucho FS600, Bayer 120 CropScience, Monheim, Germany) were kindly provided by the Luyi Cottonseed Company Ltd., Jinan, Shandong, China.

Three treatments including monoseeding, double seeding and cluster seeding were established by sowing 1 seed, 2 seeds and 10 seeds each hill using the hill-dropping seeding method (Dai et al., 2014b) (Fig. 1). A randomized complete block design with three replications was used in the experiment. Each plot consisted of six rows with row length of 15 m and in-row spacing of 76 cm. The sowing density of this experiment was 90,000 hills ha^{-1}.

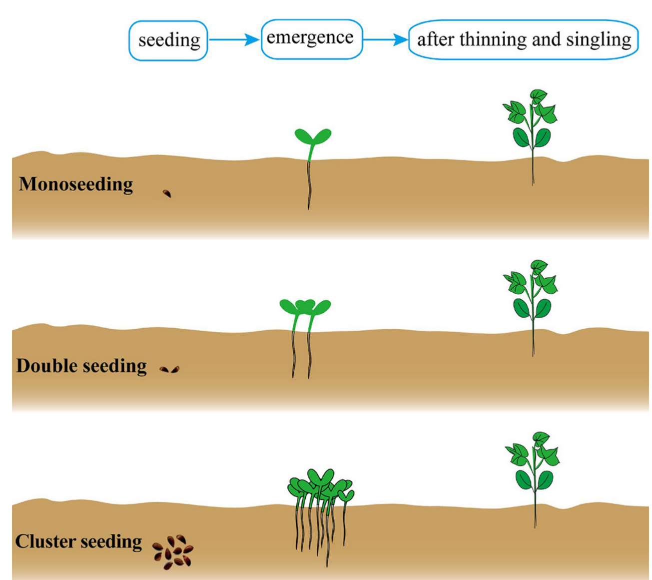

Fig. 1. Seeding, emergence and the resulting seedlings after thinning and singling under different seeding patterns.